

from 1976-2002 were selected to sequence the envelope (E) gene for identifying specific sequence patterns which may correlate with disease severity and for assessing potential trends in molecular evolution and epidemiology of circulating DEN-4 viruses. Phylogenetic analysis revealed that the majority collected in the past 27 years comprised genotype I (47 cases), 5 of the 6 more recent isolates comprised a genotype never previously described. This newly discovered genotype was associated with DF (2 cases) and DHF/DSS (3 cases). No specific sequence differences were identified between DF and DHF/DSS isolates, suggesting that the E gene alone did not determine disease severity. The phylogenetic tree revealed that genotype I of DEN-4 virus appears to have become extinct beyond 1999 and has been replaced by new lineages that evolved locally, rather than having been introduced. However, it remains unclear whether this replacement represents a selection event, so that strains differ in fitness, or a random population bottleneck. One case of the genotype IIA appears to have been introduced in 2000 from neighboring Malaysia/Indonesia. However, it appears that this genotype could not be sustained. Sequencing of the entire virus genome of selected specimens is underway to further ascertain the molecular basis to any differences in fitness among strains.

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MOLECULAR EVOLUTION AND EPIDEMIOLOGY OF DENGUE-3 AND -4 VIRUSES IN BANGKOK, THAILAND

Zhang C, Klungthong C, Monkongdee P, Mammen MP Jr and Holmes EC

Sixty dengue-3 (DEN-3) and fifty-three dengue-4 (DEN-4) virus isolates from children in Bangkok, Thailand, admitted with varying degree of dengue severity [dengue fever/dengue hemorrhagic fever/dengue shock syndrome (DF/DHF/DSS)] from 1974 to 2002 were selected to sequence the envelope (E) gene for identifying specific sequence patterns which may correlate with disease severity and for assessing potential trends in molecular evolution and epidemiology of circulating DEN-3 and -4 viruses within Bangkok. No specific sequence differences distinguished the DF and DHF/DSS for both DEN-3 and -4 was observed, suggesting that the E gene alone was not a determinant of disease severity. The phylogenetic trees showed that only genotype II of DEN-3 virus circulated in Bangkok during near past three decades and there is a clear separation within the large Thai group of viruses sampled from 1996 onwards being separated from the rest by a branch with 100% bootstrap support; and that the majority DEN-4 collected in the past 27 years comprised genotype I (47 cases), 5 of the 6 more recent isolates comprised a genotype never previously described. This newly discovered genotype was associated with DF (2 cases) and DHF/DSS (3 cases). The phylogenetic analysis revealed that the strains of DEN-3 virus prior to 1996 and DEN-4 viruses of genotype I beyond 1999 circulating in Bangkok appears to have become extinct and been replaced by new lineages that evolved locally, rather than having been introduced. These suggest that there have been a selective replacement of strain through time. One case of the genotype IIA for DEN-4 appears to have been introduced in 2000 from neighboring Malaysia or Indonesia where this genotype is known to circulate, but this genotype could not be sustained. It remains unclear and requires further study as to whether this replacement represents a selection event, so that strains differ

in fitness, or a random population bottleneck. Sequencing of the entire virus genome of selected specimens is underway to ascertain the molecular basis to any differences in fitness among strains and to further analyze selection pressure and recombination for DEN-4.

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MOLECULAR EVOLUTION AND EPIDEMIOLOGY OF DENGUE-3 AND -4 VIRUSES IN BANGKOK, THAILAND

Zhang C, Klungthong C, Monkongdee P, Mammen MP Jr and Holmes EC

Sixty dengue-3 (DEN-3) and fifty-three dengue-4 (DEN-4) virus isolates from children in Bangkok with varying degrees of clinical dengue severity [dengue fever/dengue hemorrhagic fever/dengue shock syndrome (DF/DHF/DSS)] from 1973 to 2002 were selected to sequence virus envelope (E) and partial pre-membrane/membrane (preM/M) genes. An analysis of the results was completed to observe potential trends in molecular evolution and the epidemiology of circulating dengue viruses in Bangkok, Thailand. A new genotype of DEN-4 was defined in 5 of fifty-three isolates. This new genotype caused both DF (2 cases) and DHF/DSS (3 cases). Phylogenetic analysis showed that only genotype II of the DEN-3 virus serotype and genotype I (majority), IIA (1 case) and III (we defined) of the DEN-4 (5 cases) circulated in Bangkok, Thailand during the past three decades. There were no diagnostic sequence differences between DF and DHF/DSS, suggesting that E gene alone does not determine disease severity. The Phylogenetic trees indicated that strains of DEN-3 and the genotype I of DEN-4 virus circulating in Bangkok prior to 1990 appear to have disappeared and have been replaced by new lineages that have evolved locally, rather than being introduced. However, the genotype IIA of DEN-4 seems being introduced from the neighboring country of Malaysia. Sequencing virus entire genome of some selected samples for both DEN-3 and DEN-4 is undertaking for further analysis on selection pressure and recombination.

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MOLECULAR EVOLUTION AND EPIDEMIOLOGY OF DENGUE-3 VIRUSES IN BANGKOK, THAILAND

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Sixty dengue-3 viruses (DEN-3) isolated from children admitted between 1974 and 2002 in Bangkok, Thailand, with varying degrees of dengue [dengue fever/dengue hemorrhagic fever/dengue shock syndrome (DF/DHF/DSS)] were selected for sequencing of the envelope (E) gene to identify specific sequence patterns which may correlate with disease severity. Additionally, potential trends in molecular evolution and epidemiology of circulating DEN-3 viruses within Bangkok were explored. Phylogenetic analysis indicated that only genotype II circulated in